EXHIBIT A

PubMed

Search: "Infect Immun"[Journal] AND 1999[PDAT] AND 67[VOL] AND 10[ISS] AND 5124-32[PAGE]

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We found 1 article in Infect Immun 1999:

Infect Immun. 1999 Oct;67(10):5124-32.

Local and systemic neutralizing antibody responses induced by intranasal immunization with the nontoxic binding domain of toxin A from Clostridium difficile.

Ward SJ, Douce G, Dougan G, Wren BW.

Microbial Pathogenicity Research Group, Department of Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, West Smithfield, London EC1A 7BE, United Kingdom.

Abstract

Fourteen of the 38 C-terminal repeats from Clostridium difficile toxin A (14CDTA) were cloned and expressed either with an N-terminal polyhistidine tag (14CDTA-HIS) or fused to the nontoxic binding domain from tetanus toxin (14CDTA-TETC). The recombinant proteins were successfully purified by bovine thyroglobulin affinity chromatography. Both C. difficile toxin A fusion proteins bound to known toxin A ligands present on the surface of rabbit erythrocytes. Intranasal immunization of BALB/c mice with three separate 10-microg doses of 14CDTA-HIS or -TETC generated significant levels of anti-toxin A serum antibodies compared to control animals. The coadministration of the mucosal adjuvant heat labile toxin (LT) from Escherichia coli (1 microg) significantly increased the anti-toxin A response in the serum and at the mucosal surface. Importantly, the local and systemic antibodies generated neutralized toxin A cytotoxicity. Impressive systemic and mucosal anti-toxin A responses were also seen following coadministration of 14CDTA-TETC with LTR72, an LT derivative with reduced toxicity which shows potential as a mucosal adjuvant for humans.

PMID: 10496886 [PubMed - indexed for MEDLINE] PMCID: PMC96861 Free PMC Article

Publication Types, MeSH Terms, Substances, Grant Support

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J Infect Dis. 2003 Sep 1;188(5):753-8. Epub 2003 Aug 11.

Transcutaneous immunization with tetanus toxoid and mutants of Escherichia coli heat-labile enterotoxin as adjuvants elicits strong protective antibody responses.

Tierney R, Belgnon AS, Rappuoli R, Muller S, Sesardic D, Partidos CD.

Division of Bacteriology, National Institute for Biological Standards and Control, Potters Bar, United Kingdom.

Abstract

In this study, the adjuvanticity of 2 nontoxic derivatives (LTK63 and LTR72) of heat-labile enterotoxin of Escherichia coli (LT) was evaluated and was compared with that of a cytosine phosphodiester-guanine (CpG) motif, after transcutaneous immunization with tetanus toxoid (TT). TT plus LTR72 elicited the strongest antibody responses, compared with those elicited by the other vaccines (TT, TT plus LTK63, TT plus CpG, and TT plus LTK63 plus CpG); it neutralized the toxin and conferred full protection after passive transfer in mice. Preexisting immunity to LT mutants did not adversely affect their adjuvant potency. Both LTK63 and LTR72 promoted the induction of IgG1 antibodies. In contrast, mice receiving either CpG motif alone or CpG motif plus LTK63 produced strong IgG2a anti-TT antibody responses. Overall, these findings demonstrate that mutants of enterotoxins with reduced toxicity are effective adjuvants for transcutaneous immunization.

PMID: 12934192 [PubMed - indexed for MEDLINE]

Vaccine. 2007 Apr 20;25(16):3219-27. Epub 2007 Feb 5.

Enhancement of intranasal vaccination with recombinant chain A ricin vaccine (rRV) in mice by the mucosal adjuvants LTK63 and LTR72.

Kende M, Tan X, Wlazlowski C, Williams R, Lindsey C, Del Giudice G.

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Abstract

Intranasal (i.n.) vaccination of mice with three doses of 40 microg of rRV stimulated low anti-ricin ELISA and neutralizing antibody responses, which were only marginally protective against aerosol-delivered 5-10 LD(50) of ricin toxin. To enhance the protection, and to reduce the lung injury of vaccinated mice that survived ricin toxin challenge, the mucosal adjuvant LTK63 or LTR72, two mutants of Escherichia coli LT enterotoxin adjuvant was administered with rRV. The safety of intranasally administered LTR63 was assessed as well. With 4, 2, or 1 microg of LTR63, the anti-ricin ELISA serum immunoglobulin geometric mean titer (GMT) increased up to 147-, 356-, 493-, and 17-fold for IgG, IgG1, IgG2a, and IgA, respectively. The comparable increases for GMTs of IgG and IgG1 in the presence LTR72 were up to 147-, and 617-fold, respectively. All three dose levels of LTK63 enhanced the ELISA GMTs in the lung lavage up to 192-, 22-, 4-, and 5-fold for IgG, IgG1, IgG2a, and IgA, respectively. Compared to GMT of rRV alone, the serum-neutralizing antibody GMTs for the three dose levels were enhanced up to 11-fold with LTK63. LTK63 augmented the ricin-related lymphoproliferative response of the cultured spleen lymphocytes and of the isolated CD4+ T lymphocytes. In the cultured lymphocytes, LTK63 stimulated predominantly TH1 cytokines. While only 10% of the mice that were vaccinated with rRV survived lethal challenge, in the presence of LTK63 or LTR72, the respective survival rates were augmented to 100%. Compared to the surviving mice vaccinated with rRV alone, the vaccine with LTK63 or LTR72 did not attenuate the extent of the ricin-related lung injury at a single or two time-points, respectively. Safety of LTK63 administration was indicated by the absence of histopathological changes in every organ, including the lungs and in the central nervous systems (CNS) of the mice during the entire 92 days of the study. In the nasal passages of the mice that received LTK63, a transient inflammation occurred without permanent epithelial changes. Administration of three dose levels of the adjuvant in the presence of rRV caused no additional changes. LTK63 and LTR72 both were very effective and safe mucosal adjuvants at all three dose levels employed in these studies. Both significantly enhanced the protection of a marginally effective dose of rRV against aerosol-delivered ricin challenge. LTK63 stimulated cytokines, which could be surrogate markers of efficacy, with human relevance potential. In spite of the better efficacy, rRV with LTK63, or with LTR72, failed to reduce the ricin-related lung injury. Most likely, a larger than suboptimal dose could resolve the lung injury of the vaccinated mice in the presence of a larger dose of the mucosal adjuvant.

PMID: 17343960 [PubMed - indexed for MEDLINE]

PubMed Search: "Vaccine"[Journal] AND 2002[PDAT] AND 20[VOL] AND 21-22[ISS] AND 2671-9[PAGE]

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EXHIBIT D

FULL-TEXT ARTICLE

Display Settings: Abstract

We found 1 article in Vaccine 2002:

Vaccine. 2002 Jun 21;20(21-22):2671-9.

Epidermal powder immunization using non-toxic bacterial enterotoxin adjuvants with influenza vaccine augments protective immunity.

Chen D, Endres RL, Erickson CA, Maa YF, Payne LG.

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Abstract

The non-toxic B subunit of cholera toxin (CTB) and E. coli heat-labile toxin mutant proteins with reduced toxicity (LTR72) or no toxicity (LTK63) were used as adjuvants for epidermal powder immunization (EPI) with an influenza vaccine. When administered by EPI, CTB, LTR72 and LTK63 significantly augmented antibody responses to the influenza vaccine and protection against a lethal challenge in a mouse model. The antigen dose could be reduced by 125-fold. These adjuvants were well-tolerated both locally and systemically following EPI. These results suggest that EPI with influenza vaccine and a non-toxic bacterial enterotoxin hold promise for human vaccination.

PMID: 12034092 [PubMed - indexed for MEDLINE]

MeSH Terms, Substances

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Jpn J Infect Dis. 2000 Jun;53(3):98-106.

A proposal for safety standards for human use of cholera toxin (or Escherichia coli heat-labile enterotoxin) derivatives as an adjuvant of nasal inactivated influenza vaccine.

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Abstract

Cholera toxin (CT) and Escherichia coli heat-labile toxin (LT) are not only the causative agents of diarrhea but are also strong mucosal adjuvants which enhance immune responses to mucosally coadministered bystander antigens. One of the most promising applications of these toxins would be as mucosal adjuvant of nasal influenza vaccine. In comparison to current inactivated vaccines, the nasal vaccine provides superior cross-protection by inducing production of cross-reacting anti-viral IgA antibodies in the respiratory tract even when the vaccine strain is different from the epidemic strain. On the use of the toxins as mucosal adjuvants in humans, toxicity and allergenicity of the toxins are problems which impinge on safety. To resolve these problems, various approaches have been attempted to produce less toxic and less allergenic CT (or LT) derivatives. We now propose the following standards for human use of safer CT (or LT) derivatives as an adjuvant of a nasal influenza vaccine. Thus, CT (or LT) derivatives can be administered intranasally together with a current inactivated influenza vaccine, provided they meet the following criteria. 1) A single dose of the derivatives, administered intranasally by spraying, should be around 100 Eg/adult in a volume of less than 0.5 ml. 2) CT (or LT) derivatives should retain the properties of the native CT (or LT), i. e., the ability to augment secretory IgA and serum IgG Ab responses to viral surface glycoproteins, when administered intranasally together with an inactivated influenza vaccine. 3) CT (or LT) derivatives should not induce IgE Ab responses to the vaccine, as well as to the CT (or LT) itself. 4) The CT (or LT) should be nontoxic; the toxicity of the derivatives, as determined by the Y-1 adrenal cell assay, should not exceed 1/100 EC(50) of the native CT (or 1/1000 ECi of the native CT). 5) CT (or LT) derivatives should not cause serious disease in guinea pigs when administered intranasally or intraperitoneally at the dose used in humans (around 100 Eg).

PMID: 10957706 [PubMed - indexed for MEDLINE] Free Article